

Impacts of Aging on In Vivo and In Vitro Measurements of Soil-Bound Polycyclic Aromatic Hydrocarbon Availability

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Ingestion of contaminated soil is an exposure pathway at approximately one-half of the Superfund sites in the United States. This study was designed to evaluate the impacts of aging in soil on the availability of polycyclic aromatic hydrocarbons (PAHs). Two coal tar (CT)-amended soils were prepared. One was aged for 270 days and the other was not aged. Both of these treatments were incorporated into pellets and fed to male Fischer 344 rats. Excretion of 1-hydroxypyrene (1-OHP) in urine and PAH concentrations in the liver were monitored as end points. Additionally, soil:water partitioning and desorption were measured as comparisons to the in vivo results. After 5 days of ingesting their respective treatments, rats in the aged soil group excreted 4.41 ± 1.67 ppm 1-OHP/mg of pyrene ingested while rats in the unaged soil group excreted 5.27 ± 1.37 ppm/mg of pyrene ingested. Animals fed aged CT soil had 0.051 ± 0.011 ppm carcinogenic PAHs in livers/mg ingested while rats fed unaged CT soil had 0.063 ± 0.037 ppm carcinogenic PAHs in livers/mg ingested. Partitioning and desorption results revealed a similar results. These results indicate that, at high application rates, soil contact time may not play as significant a role in determining availability as simple dispersion and sorption on soil.

Introduction

Adding a factor to adjust for bioavailability in risk assessments of soil-borne organic contaminants could reduce the time required for cleanup activities and reduce associated costs. Such an allowance is not possible at the current time because no accepted methodology for measuring availability exists. It has been shown repeatedly that soil contaminants are less

than 100% available and that they can become less available over time (1–3). Recently, a great deal of attention has focused on development of in vitro extraction methods designed to mimic digestion as a means of estimating bioavailability.

Simulated digestive tract solutions were first applied to metals, specifically, iron in food (4). Ruby and co-workers (5) used similar techniques for lead in soil and then compared these results to those obtained with a rabbit feeding study. A strong correlation between the two approaches showed that in vitro approaches could approximate bioavailability for metals; however, such success with organic compounds has proved elusive. Hack and Selenka (6) developed an in vitro method for measuring the release of PAHs and PCBs from soil and a variety of other source materials into the digestive fluids of polychaete worms. This approach incorporated the animals' stomach fluid, intestinal fluid, and food to determine the fate of nonpolar organics in the digestive tract.

Many researchers have demonstrated that PAHs are not 100% available even when dosing via gavage in corn oil (7). Thus, it is not surprising that PAHs in complex mixtures and on soil are also not 100% bioavailable upon ingestion. Koganti and co-workers (8) found that PAHs from manufactured gas plant residue are less than 100% available to mice following ingestion on soil. They estimated availability to be 76% or less based on DNA adduct formation and urinary excretion of the metabolite, 1-hydroxypyrene (1-OHP). As a companion to the study described in this paper, Bordelon and co-workers (9) fed soil spiked with coal tar in aged and unaged forms to Fischer 344 rats and measured DNA damage through ³²P-postlabeling. They found nonsignificant differences in adduct levels between rats fed aged and unaged CT-amended soils. However, a group given control coal tar spiked onto an inert carrier exhibited higher levels of DNA adducts than either of the soil groups, demonstrating the ability of soil to limit bioavailability.

This study was conducted using application rates frequently encountered in field situations. The objective of the study was to compare the availability of a complex mixture of PAHs on a freshly spiked soil and a soil aged for 270 d using both in vivo and in vitro methods. In vivo methods measured excretion of 1-OHP in the urine and accumulation of parent compounds in the liver of adult male Fischer 344 rats. In vitro methods measured partitioning of PAHs between soil and one of three aqueous solutions and desorption with Tenax TA beads.

Materials and Methods

Methanol, methylene chloride, hexane, and acetone (all ACS reagent grade) were purchased from Fisher Scientific (Pittsburgh, PA). PAH standards for chemical analysis were purchased from Supelco (Bellefonte, PA). Aryl sulfatase/ β -glucuronidase (347 000 units/g from *Helix pomatia*) and 1-OHP (98%) were purchased from Sigma (St. Louis, MO). Hydrochloric acid, acetic acid, sodium acetate, and mono- and dibasic sodium phosphates were purchased from VWR (Suwanee, GA). A total of 18 M Ω of distilled, deionized water was used for the aqueous extraction and desorption fluids unless otherwise noted.

A sample of coal tar (CT) was collected in precleaned glass jars from a closed manufactured gas plant facility in the central United States and stored at 5 ± 2 °C. Weswood silty clay loam soil (pH 7.8; 1.7% organic carbon, 12% sand, 49% silt, and 39% clay) was obtained from a fallow field on Texas A&M property on the west side of the Brazos River.

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Two aliquots of this soil were prepared. The soil was passed through a 1000- μ m brass sieve with agitation and dried overnight at 60 °C. Sterile distilled water was added to bring the soil to 18% moisture (field capacity). The wetted soil was autoclaved at 120 °C and 20 atm for 45 min in a foil-covered stainless steel pan. Six kilograms of soil was then spiked with 300 g of CT dissolved in 600 mL of methylene chloride and troweled thoroughly with sterile implements to give a final concentration of 5% CT (wt/wt). The soil was allowed to air-dry in order to remove the methylene chloride. The first aliquot of contaminated soil was stored for 270 d before use, and a second was prepared in the same manner within 24 h before use. Soils were aged in a greenhouse equipped with a heater to maintain a minimum temperature of 18 °C. The maximum temperature was maintained at the ambient outdoor temperature with a cooling fan. During aging, the highest outdoor temperature was 36 °C. The average daily high in the greenhouse for this period was 30.6 ± 6.7 °C.

Feed was prepared as described by Bordelon et al. (9). Briefly, CT-contaminated soil was added to powdered rat chow (Purina Mills, St. Louis, MO) at a level of 7% (wt/wt). This concentration gave an overall level of 0.35% CT in the diet. A 1:1:1 (by weight) mixture of gum tragacanth, sodium alginate, and methylcellulose was added to the dry feed:soil mixture at a level of 2.5 wt %. This mixture was wetted with sterile distilled, deionized water and mixed to form a paste. An autoclaved 2 cm i.d. glass tube was then inserted, and the paste was drawn into the tube with vacuum. Following this, the contents were extruded onto an autoclaved foil pan, sliced into 2.5–3-cm sections, and dried at 40 °C.

In addition to the aged CT feed (CT aged) and the freshly spiked CT feed (CT unaged), an unamended control feed and a control feed containing clean soil were prepared. A coal tar control (CT control) was also made in which the coal tar was suspended on a 3:1 mixture of SiO₂:TiO₂ rather than soil. In this situation, the CT was spiked on this inert vehicle at the same concentration and in the same manner as it was spiked on the soil. The SiO₂:TiO₂ matrix was used as a control because this material provides minimal capacity for sorption of PAHs. All prepared feeds, including both coal tar soils, clean soil, clean feed, and coal tar on SiO₂:TiO₂ were extracted 3 times with methylene chloride in a Soxtec automated Soxhlet extraction system (Tecator, Hoganas, Sweden). Extracts were cleaned up with silica gel cartridges prior to analysis.

Sixty-day-old male Fischer 344 rats weighing 210–230 g each were housed as described in Bordelon et al. (9) with the following changes. Rats were randomly assigned to treatments in groups of five. From day 0 to day 4, rats were housed in polycarbonate cages and given treatment or control feeds along with water ad libitum. From day 5 until day 11, rats were housed in metabolism cages and given clean feed, and their urine was collected and frozen upon excretion with the aid of a refrigerated metabolism cage rack. On day 12, the rats were returned to their previous cages and administered their original treatments. On day 18, the rats were asphyxiated with CO₂, and their livers were removed and frozen with liquid nitrogen for later analysis. This pattern was used in order to have samples that represent the situation when concentrations would be maximized in the urine and livers. This feeding protocol was used to allow the rodents 6 d to metabolize and excrete PAHs in the feed. After the collection of urine samples on day 12, the original feed was administered to optimize conditions for the detection of PAHs in tissues.

Three aqueous extraction solutions were employed in this study. All extractions were performed in triplicate using sterilized, glass, 500-mL screw-top bottles incubated at 22 ± 2 °C and shaken at 100 rpm. Extraction solutions were 0.2 M phosphate-buffered water (pH 7), distilled water, and an

acetic acid solution at pH 2.8. For all methods, 20 g of soil (dry weight) was extracted with 200 mL of solution. For the distilled water extraction, sterile distilled water was added to each of three autoclaved bottles along with soil. Samples were shaken for 24 h. For the phosphate buffer extraction, a 0.2 M solution of mono- and dibasic sodium phosphates in distilled water adjusted to pH 7 was autoclaved and added to each of three autoclaved bottles along with soil. Samples were shaken for 24 h. For the acetic acid extraction, a solution based on the U.S. EPA's Toxicity Characteristic Leaching Procedure (TCLP) (10) fluid composed of acetic acid in deionized water at pH 2.8 was autoclaved and combined with soil in each of three autoclaved bottles. Samples were shaken 24 h. K_d values were calculated by dividing the concentration in a given aqueous solution by the concentration remaining in the soil after the 24-h equilibration.

When shaking was complete, samples were centrifuged for 1 h at 600g and filtered through a Whatman No. 42 paper filter (Whatman Paper Ltd., Maidstone, England) in a Buchner funnel with vacuum to remove suspended solids. The filtered solution was extracted with Bakerbond C-18 cartridges (JT Baker, Phillipsburg, NJ).

The methods of Cornelissen et al. (11) for measuring desorption of nonpolar organics in distilled water with 60–80-mesh Tenax TA beads were used with the following modifications. Experiments were carried out in triplicate in 30-mL amber glass vials. Vials containing 20 mL of sterile distilled water, 200 mg of Tenax TA beads (Supelco, Bellefonte, PA), and approximately 1 g of soil were shaken horizontally at 200 rpm at 25 ± 2 °C for 9 d. Beads were removed at 1, 3, 5, 24, 72, 120, and 216 h and replaced with another 200 mg of clean beads. After being shaken, the vials were centrifuged at 600g for 15 min to settle the soil as a pellet, leaving the beads suspended in the supernatant. The beads and supernatant were decanted through a 140-mesh hexane-rinsed brass sieve. The supernatant was set aside, and the remaining beads were washed from the vial with distilled water. The original water was poured back into the vials to prevent losses of any fine suspended sediment or dissolved organic carbon, and fresh Tenax was added. Following recovery, the beads were sonicated for 10 min to improve recovery and then extracted with three 10-mL rinses of 1:1 hexane:acetone.

Data were fit using Table Curve software (Jandel Scientific, San Rafael, CA) to the natural logarithm of

$$S_t/S_0 = C_L e^{-k_1 t} + C_R e^{-k_2 t} \quad (1)$$

where S_t/S_0 is the concentration in the soil at time t over the concentration in the soil at time 0; C_L and C_R (unitless) are the proportions in the labile and refractory phases, respectively. The terms k_1 and k_2 (h⁻¹) are rate constants for release from each of the two compartments, and t is time of desorption (h).

Liver samples were freeze-dried at –65 °C and 15 mTorr for 10–15 h in an FD-1-84A freeze-dryer (FTS Systems, Stone Ridge, NY). After being dried, livers were ground and then extracted twice in a Tecator automated Soxhlet device (Tecator, Hoganas, Sweden) with a solution of methylene chloride:tetrahydrofuran (9:1). Extracts were concentrated to 1 mL and cleaned up using a column of 10 g of 5% deactivated 100–200-mesh silica over 20 g of 1% deactivated 80–200-mesh alumina. A fraction containing PAHs was eluted with 200 mL of hexane. After reducing the volume to 1 mL, this extract was further cleaned up using gel permeation chromatography. The column was a 21.2 mm \times 300 mm Phenogel 100 A column (Phenomenex Torrance, CA). The mobile phase was methylene chloride at 5 mL/min. PAHs were collected between 12 and 16 min into the run.

TABLE 1. Concentrations of Polycyclic Aromatic Hydrocarbons in Soil and Inert Carrier Prior to Feed Preparation ($\mu\text{g/g}$)^b

compound	CT unaged	CT aged	CT control	clean soil	clean feed
anthracene	83.9 \pm 20.5	79.8 \pm 29.9	97.5 \pm 9.9	nd	nd
phenanthrene	191.2 \pm 7.6	223.5 \pm 62.8	231.2 \pm 20.5	nd	nd
fluoranthene	140.4 \pm 6.9	166.7 \pm 56.9	140.8 \pm 10.2	nd	nd
pyrene	145.7 \pm 4.6	177.7 \pm 56.1	151.2 \pm 11.6	nd	nd
benz[a]anthracene ^b	144.7 \pm 23.6	160.3 \pm 56.2	147.7 \pm 19.7	nd	nd
chrysene ^b	127.2 \pm 8.6	129.3 \pm 61.4	100.4 \pm 10.1	nd	nd
benzo[b]fluoranthene ^b	80.0 \pm 7.2	76.6 \pm 21.3	71.3 \pm 5.2	nd	nd
benzo[k]fluoranthene [*]	36.8 \pm 1.7	31.4 \pm 14.2	27.2 \pm 2.5	nd	nd
benzo[a]pyrene ^b	84.7 \pm 3.0	79.7 \pm 25.0	76.4 \pm 6.4	nd	nd
indeno[1,2,3-cd]pyrene ^b	44.5 \pm 5.6	43.6 \pm 14.2	40.5 \pm 2.9	nd	nd
dibenz[a,h]anthracene ^b	15.1 \pm 2.4	14.6 \pm 4.9	14.0 \pm 1.1	nd	nd
benzo[g,h,i]perylene	35.3 \pm 4.1	34.7 \pm 10.7	31.9 \pm 2.4	nd	nd
carcinogenic PAHs	533.0 \pm 57.7	535.5 \pm 168.4	477.5 \pm 37.1	nd	nd

^a CT, coal tar. CT unaged, soil spiked with coal tar (CT) shortly before use. CT aged, soil spiked with CT 270 d before use. CT control, 3:1 SiO₂:TiO₂ spiked with CT shortly before use. Clean soil, unspiked soil. Clean feed, feed as received from supplier. nd, not detected. Results were compared using one-way analysis of variance followed by Tukey's test. ^b PAHs for which the U.S. EPA provides a cancer slope factor.

TABLE 2. Concentration of Polycyclic Aromatic Hydrocarbons in Prepared Feeds ($\mu\text{g/g}$)^a

compound	CT unaged	CT aged	CT control	clean soil	clean feed
anthracene	7.1 \pm 0.9	4.4 \pm 0.8	3.3 \pm 0.1	nd	nd
phenanthrene	34.7 \pm 1.3 ^A	25.3 \pm 6.5 ^B	13.1 \pm 1.0 ^{A,B}	nd	nd
fluoranthene	18.1 \pm 0.9 ^C	15.2 \pm 4.5 ^D	7.1 \pm 0.6 ^{C,D}	nd	nd
pyrene	22.0 \pm 0.7 ^E	16.6 \pm 4.9	8.0 \pm 0.6 ^E	nd	nd
benz[a]anthracene ^b	14.8 \pm 0.6 ^{F,G}	7.8 \pm 3.3 ^F	4.4 \pm 0.3 ^G	nd	nd
chrysene ^b	12.6 \pm 1.4 ^{H,I}	6.7 \pm 2.1 ^H	4.4 \pm 0.7 ^I	nd	nd
benzo[b]fluoranthene ^b	6.9 \pm 0.8 ^J	5.2 \pm 1.5	3.0 \pm 0.1 ^J	nd	nd
benzo[k]fluoranthene ^b	2.6 \pm 0.3 ^{K,L}	1.5 \pm 0.4 ^{K,M}	0.6 \pm 0.1 ^{L,M}	nd	nd
benzo[a]pyrene ^b	6.7 \pm 0.8 ^{N,O}	4.5 \pm 1.2 ^N	2.8 \pm 0.1 ^O	nd	nd
indeno[1,2,3-cd]pyrene ^b	3.2 \pm 0.3 ^P	2.6 \pm 0.7 ^Q	1.4 \pm 0.1 ^{P,Q}	nd	nd
dibenz[a,h]anthracene ^b	1.1 \pm 0.2 ^R	0.9 \pm 0.3	0.5 \pm 0.0 ^R	nd	nd
benzo[g,h,i]perylene ^b	2.7 \pm 0.3 ^S	2.2 \pm 0.6 ^T	1.1 \pm 0.1 ^{S,T}	nd	nd
carcinogenic PAHs	47.9 \pm 4.7	29.2 \pm 4.9	17.1 \pm 1.5	nd	nd

^a CT, coal tar. CT unaged, feed containing freshly spiked soil. CT aged, feed containing aged spiked soil. CT control, feed containing inert vehicle spiked with CT. Clean soil, feed containing clean soil. Clean feed, feed with no amendments. nd, not detected. Results by treatment group were compared using one-way analysis of variance followed by Tukey's test. Values in the same row that are followed by the same superscript letter are significantly different from one another at $p < 0.05$. ^b PAHs for which the U.S. EPA provides a cancer slope factor.

Liver, shake flask, and desorption experiment samples were analyzed with an HP-5890 GC coupled to an FID detector. The column was a 30-m DB5 MS fused silica megabore (0.53 mm i.d.) column (J&W Scientific, Folsom, CA). The temperature program began at 100 °C for 4 min, ramped 8 °C/min to 280 °C, and was held for 34 min. The inlet was kept at 250 °C, and the detector was kept at 325 °C. Incomplete resolution of benzo[b]fluoranthene from benzo[k]fluoranthene and of indeno[1,2,3-cd]pyrene from benzo[g,h,i]perylene necessitated that these compounds be presented as a sum when determined by GC/FID. Extracts of the feeds, soils, and CT on TiO₂:SiO₂ were analyzed via an HP-5890 series II GC coupled to an HP 5972-MS. The column was a 30 m, 0.25 mm i.d. HP5 MS. The temperature program began at 35 °C for 6 min, ramped 5 °C/min to 300 °C, and was held for 30 min. All analyses were completed using the scan mode from 50 to 550 m/z .

Urine samples were processed and analyzed with the methods of Jongeneelen and co-workers (12). Briefly, each sample was adjusted to pH 5 with 10% HCl and buffered with 10 mL of 0.1 M sodium acetate. A total 25 μL of a 50 000 unit/mL solution of β -glucuronidase/aryl sulfatase solution was then added, and the samples were incubated for 20 \pm 2 h at 37 °C on a shaker at 100 rpm. Following incubation, the samples were extracted with 1-g Bakerbond C18 cartridges (JT Baker, Phillipsburg, NJ). Following extraction and concentration under a stream of nitrogen, samples were analyzed using a Waters (Milford, MA) 660 HPLC and a 996 photodiode array detector at 254 nm. A 15 cm \times 4.6 mm Supelcosil LC-PAH C-18 column (Supelco, Bellefonte, PA) heated to 40 °C

was used for the analysis. The mobile phase was 54% water:46% acetonitrile for 5 min and ramped to 19.7% water:80.3% acetonitrile by 30 min. Data were fit to

$$Y = Re^{-k_1 t} + Se^{-k_2 t} \quad (2)$$

where R is the concentration in the rapidly excreted phase (unitless), S is the concentration in the slowly excreted phase (unitless), k_1 is the rate constant for release from the rapid phase (d^{-1}), k_2 is the rate constant for release from the slow phase (d^{-1}), and t is time (d).

Data were analyzed using Sigma Stat software, version 2.03 (Jandel Scientific, San Rafael, CA). Feed consumption was analyzed using a one-way analysis of variance (ANOVA) followed by a Tukey's test with $p < 0.05$. Body weights were analyzed with a one-way ANOVA with $p < 0.05$. Liver and urine data were analyzed using a one-way ANOVA followed by a Tukey's test with $p < 0.05$. Data from in vitro measurements were analyzed using t -tests with $p < 0.05$.

Results and Discussion

Feed Preparation and Analysis. Analysis of both CT soils and the CT-amended SiO₂:TiO₂ vehicle showed no significant differences for levels of any given PAH (Table 1). PAHs were not detected in the clean soil and clean feed or when plain control and soil control feeds were analyzed. Discrepancies were found in the analyses of the CT soil feeds and CT control feeds (Table 2). CT control had lower levels of all measured PAHs than the CT soil feeds. Since there were no significant

TABLE 3. Feed Consumption by Treatment Group (g)^a

group	consumed premetabolism cage (5 d)	consumed postmetabolism cage (6 d)
CT unaged	23.04 ± 0.96 ^A	36.55 ± 20.4 ^D
CT aged	30.40 ± 5.05 ^{B,C}	41.79 ± 11.62 ^E
CT control	15.93 ± 3.01 ^C	44.17 ± 28.05
clean soil	57.09 ± 7.54 ^{A,B,C}	80.08 ± 14.36
clean feed	59.36 ± 2.45 ^{A,B,C}	89.86 ± 5.1 ^{D,E}

^a CT, coal tar. CT unaged, feed containing freshly spiked soil. CT aged, feed containing aged spiked soil. CT control, feed containing inert vehicle spiked with CT. Clean soil, feed containing clean soil. Clean feed, feed with no amendments. Results were compared by treatment group within each period using one-way analysis of variance followed by Tukey's test. Values in the same row that are followed by the same superscript letter are significantly different from one another at $p < 0.05$.

differences between the CT containing vehicles used to make the feed, it is most likely that an error occurred during food preparation. For this reason, all data are presented on a per milligram of PAH ingested basis. Because of the discrepancy in PAH content of the feeds and some possible aversion to the scent or taste of coal tar on the pellets, CT control rats consumed an amount of food that was significantly less than control rats (Table 3). Consumption among rats from CT-fed groups in general was less than that among non-CT groups. Presumably, this is a result of a smell or taste associated with CT. None of the treatment groups, however, had final body weights that were significantly different from animals in the soil control group. The final body weight for rats administered the CT unaged (214.5 ± 14.9 g) and CT control (218.6 ± 14.2 g) feed were significantly lower than rats given plain control feed (261.1 ± 2.2 g).

Analyses of the aged and unaged CT soils revealed greater variability in the aged sample. This may be a result of partial degradation during the 270-day aging period. Alternatively, the variability of the aged soil may reflect a slight partitioning of the hydrocarbons into the soil. As stated previously, no significant differences are seen among the CT soil and the CT SiO₂:TiO₂ samples. Levels of all PAHs were significantly lower in the CT control feed, however, probably as a result of heterogeneity. This error was compensated for by presenting data on a per milligram ingested basis. Since there are no significant differences between CT treatment groups and the soil control, it was assumed that an adjustment for body weight was not necessary.

Hydroxypyrene in Urine. Analyses of urine samples found no detectable 1-OHP from plain control and soil control animals. Figure 1 presents data from urinary analyses fit to the biexponential decay model in eq 2. Urine from CT control animals had a significantly larger concentration of 1-OHP/mg of pyrene ingested than the other two CT-fed groups.

Aging had no significant impact on 1-OHP excretion when comparing CT unaged and CT aged groups (Figure 1). For the 5-day sampling period, the concentration of 1-OHP in urine/mg of pyrene ingested from CT unaged and CT aged rats was not significantly different. The concentration of 1-OHP in urine from both CT soil groups were significantly different from CT control rats on days 1 and 2 at $p < 0.05$.

Bouchard and Viau (13) and Withey and co-workers (14) found that excretion of 1-OHP is dose-dependent; a larger dose leads to a faster rate of release. This pattern was observed in the current study. In urine from CT control rats, there was an initial rapid loss of 1-OHP while in the CT soil rats a much slower elimination was observed. In addition, there are no significant differences in ppm 1-OHP/mg of pyrene ingested between the two CT soil groups, implying minimal effects of aging.

PAHs in Livers. Liver weights were not significantly different between treatment groups at $p < 0.05$. PAH levels in livers from each group are presented in Table 4. There were no significant differences between treatment groups for anthracene, phenanthrene, fluoranthene, pyrene, benz[a]anthracene, chrysene, and benzo[g,h,i]perylene. In the case of some larger compounds and total carcinogenic PAHs, one or both CT-amended soil groups had contaminant concentrations in the liver that were significantly different from those of the CT control animals. Levels of benzo[fluoranthene] were 0.109 ± 0.013 ppm in liver tissue/mg ingested for CT aged rats, 0.158 ± 0.085 ppm in liver/mg ingested for CT unaged rats, and 0.315 ± 0.133 ppm in liver/mg ingested for CT control rats. For the benzo[fluoranthene], both soil treatments were significantly different from CT control at $p < 0.05$. The concentration of benzo[a]pyrene in the liver of CT aged animals was 0.054 ± 0.006 ppm/mg ingested, while the benzo[a]pyrene concentration in livers from CT control animals was 0.158 ± 0.065 ppm/mg ingested. Similar results were observed with indeno[1,2,3-cd]pyrene and dibenz[a,h]anthracene. For the carcinogenic PAHs overall, both CT soil groups were significantly different from CT control at $p < 0.05$. CT aged and CT unaged had 0.051 ± 0.011 and 0.063 ± 0.037 ppm carcinogenic PAHs in liver tissue/mg ingested, respectively, while CT control rats had 0.133 ± 0.053 ppm in liver/mg ingested. Differences between CT aged and CT unaged groups were not significant for any of the PAHs examined at $p < 0.05$.

Metabolism and excretion of low molecular weight PAHs seem to have produced a trend opposite that seen in the high molecular weight compounds. Anthracene, phenanthrene, and fluoranthene are most prevalent in the livers of animals in the aged CT group, followed by freshly spiked CT, and then CT control. While none of the differences between groups are significant for any of these three PAHs, the trend

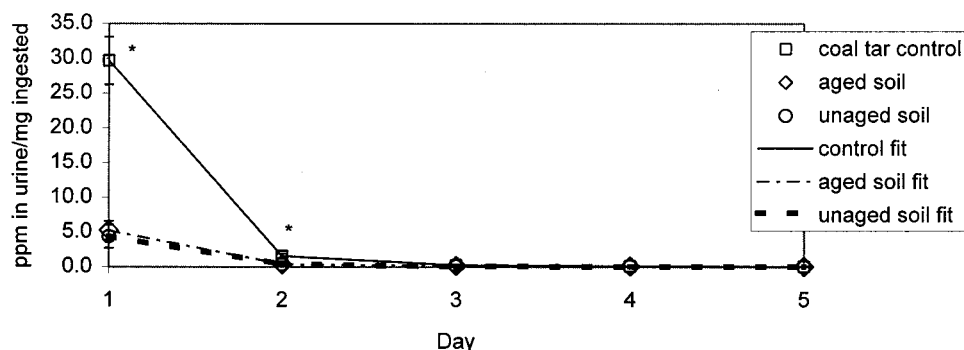


FIGURE 1. Excretion of 1-hydroxypyrene in urine from rats in unaged, aged, and coal tar control groups. Data were fit to $Y = Re^{-k_1t} + Se^{-k_2t}$. Asterisks indicate a significant difference between the coal tar control group and the other two treatment groups at $p < 0.05$. Results were compared using a one-way analysis of variance followed by Tukey's test.

TABLE 4. Concentration of Polycyclic Aromatic Hydrocarbons in Livers by Treatment Group (μg of Compound (g of Liver) $^{-1}$ (mg Ingested) $^{-1}$)^a

compound	CT unaged	CT aged	CT control	clean soil	clean feed
anthracene	0.028 \pm 0.021	0.093 \pm 0.105	0.017 \pm 0.024	nd	nd
phenanthrene	0.042 \pm 0.036	0.257 \pm 0.318	0.012 \pm 0.01	nd	nd
fluoranthene	0.108 \pm 0.074	0.137 \pm 0.08	0.081 \pm 0.046	nd	nd
pyrene	0.073 \pm 0.044	0.097 \pm 0.063	0.099 \pm 0.047	nd	nd
benz[a]anthracene ^b	0.018 \pm 0.01	0.025 \pm 0.02	0.038 \pm 0.026	nd	nd
chrysene ^b	0.021 \pm 0.01	0.03 \pm 0.027	0.043 \pm 0.041	nd	nd
benzofluoranthenes ^{b,c}	0.158 \pm 0.085 ^A	0.109 \pm 0.013 ^B	0.315 \pm 0.133 ^{A,B}	nd	nd
benzo[a]pyrene ^b	0.097 \pm 0.07	0.054 \pm 0.006 ^C	0.158 \pm 0.065 ^C	nd	nd
indeno[1,2,3- <i>cd</i>]pyrene ^{b/} dibenz[a,h]anthracene ^{b,c}	0.077 \pm 0.057	0.032 \pm 0.006 ^D	0.178 \pm 0.1 ^D	nd	nd
benzo[<i>g,h,i</i>]perylene	0.117 \pm 0.052	0.049 \pm 0.023	0.101 \pm 0.04	nd	nd
carcinogenic PAHs	0.063 \pm 0.037 ^E	0.051 \pm 0.011 ^F	0.133 \pm 0.053 ^{E,F}	nd	nd

^a CT, coal tar. CT unaged, feed containing freshly spiked soil. CT aged, feed containing aged spiked soil. CT control, feed containing inert vehicle spiked with CT. Clean soil, feed containing clean soil. Clean feed, feed with no amendments. nd, not detected. Results were compared using one-way analysis of variance followed by Tukey's test. Values in the same row that are followed by the *same* superscript letter are significantly different from one another at $p < 0.05$. ^b PAHs for which the U.S. EPA provides a cancer slope factor. ^c Compounds were summed as a result of incomplete resolution during analysis.

TABLE 5. Log K_d Values of Polycyclic Aromatic Hydrocarbons from Aged and Freshly Spiked Soils^a

compound	unaged soil/ phosphate buffer	aged soil/ phosphate buffer	unaged soil/ distilled water	aged soil/ distilled water	unaged soil/ TCLP fluid	aged soil/ TCLP fluid
anthracene	4.62 \pm 0.05	4.63 \pm 0.02	4.92 \pm 0.1 ^E	4.78 \pm 0.11 ^E	5.28 \pm 0.45	5.15 \pm 0.12
phenanthrene	4.58 \pm 0.23	4.37 \pm 0.14	4.79 \pm 0.09	4.47 \pm 0.14	5.01 \pm 0.35	4.89 \pm 0.08
fluoranthene	4.42 \pm 0.06 ^A	5.43 \pm 0.19 ^A	4.78 \pm 0.1 ^F	5.39 \pm 0.03 ^F	5.31 \pm 0.63	5.29 \pm 0.7
pyrene	4.74 \pm 0.1	4.48 \pm 0.24	5.08 \pm 0.02	4.91 \pm 0.61	5.7 \pm 0.21	5.27 \pm 0.2
benz[a]anthracene ^b	4.6 \pm 0.13 ^B	4.11 \pm 0.03 ^B	5.63 \pm 0.13	5.25 \pm 0.27	6.51 \pm 0.35	6.16 \pm 0.05
chrysene ^b	6.21 \pm 0.55	5.86 \pm 0.34	6.34 \pm 0.05 ^G	5.68 \pm 0.37 ^G	6.14 \pm 0.11 ^H	6.42 \pm 0.1 ^H
benzofluoranthenes ^{b,c}	5.69 \pm 0.32	5.97 \pm 0.05	5.84 \pm 0.11	5.79 \pm 0.28	6.03 \pm 0.1 ^I	6.72 \pm 0.2 ^I
benzo[a]pyrene ^b	6.00 \pm 0.43	5.43 \pm 0.09	6.04 \pm 0.34	5.38 \pm 0.29	5.81 \pm 0.14 ^J	6.63 \pm 0.13 ^J
indeno[1,2,3- <i>cd</i>]pyrene ^{b/} dibenz[a,h]anthracene ^{b,c}	5.14 \pm 0.02 ^C	5.84 \pm 0.12 ^C	5.64 \pm 0.14	5.71 \pm 0.43	5.86 \pm 0.17	5.52 \pm 0.26
benzo[<i>g,h,i</i>]perylene	5.5 \pm 0.14 ^D	6.2 \pm 0.12 ^D	5.78 \pm 0.31	6.18 \pm 0.3	6.33 \pm 0.36	6.71 \pm 0.08
carcinogenic PAHs	5.55 \pm 0.02	4.62 \pm 0.03	5.1 \pm 0.13	5.48 \pm 0.3	5.95 \pm 0.16 ^K	6.38 \pm 0.06 ^K

^a Phosphate buffer, 0.2 M mono- and dibasic sodium phosphates in deionized water. TCLP fluid, Toxicity Characteristic Leaching Procedure fluid (acetic acid in deionized water at pH 2.8). To determine log K_d , 20 g of each soil was shaken with 200 mL of each fluid in triplicate for 24 h. Results were compared using *t*-tests. Values in the same row that are followed by the *same* superscript letter are significantly different from one another at $p < 0.05$. ^b PAHs for which the U.S. EPA provides a cancer slope factor. ^c Compounds were summed because of incomplete resolution during analysis.

is consistent. Pyrene, benz[a]anthracene, and chrysene display no consistent trend with results from all three CT treated groups being roughly equal. Larger PAHs, encompassing everything from the benzofluoranthenes through total carcinogenic PAHs, are present in the greatest amounts in the livers of CT control rats followed by freshly spiked CT and aged CT. These size-dependent differences could result from induction of cytochrome P450 enzymes by large molecular weight PAHs. As the burden of compounds such as benzo[a]pyrene increases, the ability of the liver to oxidize and eliminate the smaller PAHs such as anthracene, phenanthrene, and fluoranthene increases, leading to a smaller amount of these compounds in the unmetabolized state. Since these animals had 6 days to ingest the CT feeds after the metabolism cages, there should have been enough time to induce oxidative enzymes and begin eliminating smaller PAHs (15). The lack of significant differences between the aged and freshly spiked CT groups indicates that aging did not limit availability as much as simply being incorporated into the soil. CT control animals often had levels of high molecular weight PAHs that were significant or at least elevated over those of the other two CT-treated groups.

Soil:Water Partitioning. Values of log K_d were generally larger for PAHs from aged soil. The differences, however, were significant in only 11 of 33 possible measurements. All three aqueous solutions used for partitioning measurements proved equally sensitive toward detecting differences be-

tween aged and unaged soils (Table 5). Use of phosphate buffer as the partitioning fluid showed significant differences between the aging treatments for a range of PAHs. Fluoranthene had a log K_d of 4.42 ± 0.06 on day 0 and 5.43 ± 0.19 on day 270. Results for indeno[1,2,3-*cd*]pyrene/dibenz[a,h]anthracene and benzo[*g,h,i*]perylene were similar. Benz[a]anthracene had a significantly lower log K_d value in the aged soil than the unaged when measured with phosphate buffer. Log K_d for benz[a]anthracene was 4.61 ± 0.14 for the unaged soil and 4.11 ± 0.02 for the aged soil. Distilled water appeared to be most effective at detecting aging effects among the small to midrange molecular weight PAHs. Fluoranthene from unaged CT soil had a log K_d of 4.78 ± 0.11 , which was significant from the value for aged soil of 5.39 ± 0.03 at $p < 0.05$. Anthracene and chrysene also had significantly different results between unaged and aged soils in distilled water. Log K_d for anthracene from unaged soil was 4.79 ± 0.09 while for aged soil the value was 4.47 ± 0.14 . In the case of chrysene, the unaged soil value was 6.34 ± 0.05 and the value for aged soil was 5.67 ± 0.37 . TCLP fluid was best at detecting significant differences among the medium to large molecular weight PAHs. In all of the cases where a significant difference was found with this solution, the unaged value was larger than the aged value. For example, benzo[a]pyrene had a value of 5.81 ± 0.14 for unaged soil and a value of 6.63 ± 0.13 for aged soil. Results for chrysene, the benzofluoranthenes, and the carcinogenic PAHs were similar.

TABLE 6. Curve Parameters for Polycyclic Aromatic Hydrocarbon Desorption Data from Aged and Unaged Soils^a

compound	unaged C_L	aged C_L	unaged k_1 (h ⁻¹)	aged k_1 (h ⁻¹)	unaged C_R	aged C_R
anthracene	0.60 ± 0.13	0.75 ± 0.33	0.89 ± 0.37	0.59 ± 0.32	0.38 ± 0.04 ^A	0.26 ± 0.06 ^A
phenanthrene	0.56 ± 0.28	0.59 ± 0.2	0.44 ± 0.41	0.46 ± 0.23	0.41 ± 0.11	0.38 ± 0.06
fluoranthene	0.57 ± 0.05	0.63 ± 0.14	0.22 ± 0.04 ^B	0.25 ± 0.09 ^B	0.4 ± 0.02	0.39 ± 0.04
pyrene	0.5 ± 0.05	0.59 ± 0.11	0.21 ± 0.04	0.23 ± 0.08	0.48 ± 0.02 ^C	0.44 ± 0.04 ^C
benz[a]anthracene ^b	0.44 ± 0.04	0.48 ± 0.08	0.05 ± 0.01	0.07 ± 0.03	0.52 ± 0.04	0.51 ± 0.06
chrysene ^b	0.43 ± 0.05 ^D	0.46 ± 0.07 ^D	0.05 ± 0.02 ^E	0.07 ± 0.03 ^E	0.53 ± 0.04	0.51 ± 0.06
benzofluoranthenes ^{b,c}	0.24 ± 0.05	0.39 ± 0.1	0.05 ± 0.00 ^F	0.04 ± 0.02 ^F	0.73 ± 0.05	0.58 ± 0.11
benzo[a]pyrene ^b	0.24 ± 0.04	0.31 ± 0.13	0.05 ± 0.24	0.03 ± 0.03	0.75 ± 0.04	0.68 ± 0.14
indeno[1,2,3- <i>cd</i>]pyrene ^{b/} dibenz[a,h]anthracene ^{b,c}	0.13 ± 0.05	0.12 ± 0.13	0.18 ± 0.16 ^G	0.06 ± 0.17 ^G	0.87 ± 0.04	0.86 ± 0.13
benzo[g,h,i]perylene ^b	0.11 ± 0.02 ^H	0.09 ± 0.04 ^H	0.2 ± 0.11	0.22 ± 0.23	0.89 ± 0.02	0.91 ± 0.03
carcinogenic PAHs	0.34 ± 0.03	0.39 ± 0.07	0.05 ± 0.01 ^I	0.06 ± 0.03 ^I	0.63 ± 0.03	0.58 ± 0.07

^a Desorption measured with Tenax beads at 1, 3, 5, 24, 72, 120, and 219 h. Parameters were derived by fitting data to the natural logarithm of $S_t/S_0 = C_L e^{-k_1 t} + C_R e^{-k_2 t}$. Values for k_2 were on the order of 1 E^{-2} to 1 E^{-4} and were not significantly different between the two soils (data not presented). Results were compared using *t*-tests. Values in the same row that are followed by the same superscript letter are significantly different from one another at $p < 0.05$. ^b PAHs for which the U.S. EPA provides a cancer slope factor. ^c Compounds were summed because of incomplete resolution during analysis.

No consistent trend was observed among partitioning measurements between PAHs from aged and unaged soils. Eight of the 11 significant differences were between a smaller value for day 0 and a larger value for day 270. Twenty-two of the comparisons however had no significant difference between day 0 and day 270 samples. This again implies that aging for 270 d failed to have a significant impact on the availability of PAHs from CT. Values obtained by each of the solutions tended to increase in the order: phosphate buffer, distilled water, TCLP fluid. Phosphate buffer extracts organic carbon from the soil more efficiently than the other solutions as evidenced by the dark brown color of the solution following centrifugation. Therefore, it is better at extracting nonpolar organic compounds than the other two fluids and tends to give lower values of log K_d . Distilled water and TCLP fluid are similar in their extraction abilities with distilled water producing slightly lower values of log K_d .

Desorption Measurements. The majority of the values obtained in the desorption experiments were not significantly different between aged and unaged soils (Table 6). The concentration of chrysene in the labile fraction, C_L , was lower in the unaged soil than the aged soil with values of 0.41 ± 0.01 and 0.46 ± 0.02 , respectively. Benzo[g,h,i]perylene had a larger value in day 0 soil than day 270 soil, with values of 0.11 ± 0.00 and 0.09 ± 0.00 , respectively. A larger number of significant differences was observed for the rate parameter, k_1 . For fluoranthene, chrysene, and total carcinogenic PAHs, it was larger for the day 270 soil than the day 0 soil. For the benzofluoranthenes, indeno[1,2,3-*cd*]pyrene and dibenz[a,h]anthracene, this parameter was smaller in day 270 soil than in day 0 soil. Only anthracene and pyrene had statistically significant differences between C_R values for the two soils. Both of these PAHs had lower values of C_R in the day 270 sample than in the day 0 sample. K_2 was on the order of 10 E^{-2} – 10 E^{-4} for all PAHs. There were no significant differences between soils (data not presented).

Desorption measurements agree with the partitioning and in vivo data. No consistent differences were found between samples from day 0 and day 270 soils. When the value of C_L for a given PAH was significantly different between the soils, the corresponding value of C_R was not significantly different. The same was true for significant differences in values of C_R . As a result, it is difficult to say what meaning the differences have, if any. Additionally, k_1 was the only one of the two rate constants to exhibit significant differences between the two soils. For three compounds it increased and for two others it decreased. k_2 was essentially 0 for all compounds. This implies that the portion of each PAH in the refractory phase,

C_R , would be unlikely to desorb. Overall, no consistent pattern of diminished availability for PAHs from the day aged as compared to the unaged soil was evident.

Results of the current study are unique to the soil, waste, and dosages employed. At the application rate used in this study, sorption appears to have been influenced more by dissolution from the coal tar than by binding to soil particles or organic matter. The results of both in vivo and in vitro studies indicate that aging a CT on Weswood soil for 270 days had minimal impact on availability. Whereas the results of the feeding study would suggest that incorporation of the CT into the soil resulted in decreased availability as demonstrated by a reduction in 1-OHP elimination and generally lower concentrations of high molecular weight PAHs in livers. Additional studies are needed to better quantitate the availability of soil-borne PAHs from complex mixtures. Further investigation of the role that soil parameters and mixture characteristics play in determining availability would make development of an in vitro method for approximating bioavailability more feasible.

Acknowledgments

The authors acknowledge the assistance of Tim Phillips, Shawna Lemke, Lingyu He, Butch Dittfurth, and Natalie Bullion. This research was funded by U.S. EPA Grant R825408 and NIEHS Grant ES04917.

Literature Cited

- Pignatello, J. J.; Xing, B. *Environ. Sci. Technol.* **1996**, *30*, 1–11.
- Robertson, B. K.; Alexander, M. *Environ. Sci. Technol.* **1998**, *17*, 1034–1038.
- Van Schooten, F. J.; Moonen, E. J. C.; van der Wal, L.; Levels, P.; Kleinjans, J. C. S. *Arch. Environ. Contam. Toxicol.* **1997**, *33*, 317–322.
- Schricker, B. R.; Miller, D. D.; Rasmussen, R. R.; vanCampen, D. *Am. J. Clin. Nutr.* **1981**, *34*, 2257–2263.
- Ruby, M. V.; Davis, A.; Link, T. E.; Schoof, R.; Chaney, R. L.; Freeman, G. B.; Bergstrom, P. *Environ. Sci. Technol.* **1993**, *27*, 2870–2877.
- Hack, A.; Selenka, F. *Toxicol. Lett.* **1996**, *88*, 199–210.
- Foth, H.; Kahl, R.; Kahl, G. F. *Chem. Toxicol.* **1988**, *26*, 45–51.
- Koganti, A.; Spina, D. A.; Rozett, K.; Ma, B. L.; Weyand, E. H.; Taylor, B. B.; Mauro, D. M. *Environ. Sci. Technol.* **1998**, *32*, 3104–3112.
- Bordelon, N. R.; Donnelly, K. C.; King, L. C.; Wolf, D. C.; Reeves, W. R.; George, S. E. *Toxicol. Sci.* **2000**, *56*, 37–48.
- U.S. Environmental Protection Agency, Office of Solid Waste, and U.S. Department of Commerce. National Technical Information Service: Method 1311, Toxicity Characteristic Leaching Procedure in SW 846 Test Methods for Evaluating Solid Waste Physical/Chemical Methods, Washington, DC, 1996; pp 211–236.

- (11) Cornelissen, G.; van Noort, P. C. M.; Govers, H. A. J. *Environ. Toxicol. Chem.* **1997**, *16*, 1351–1357.
- (12) Jongeneelen, F. J.; Anzion, R. B. M.; Henderson, P. T. *J. Chromatogr.* **1987**, *413*, 227–232.
- (13) Bouchard, M.; Viau, C. *Toxicol. Appl. Pharmacol.* **1996**, *139*, 301–309.
- (14) Withey, J. R.; Law, F. C. P.; Endrenyi, L. *J. Toxicol. Environ. Health* **1991**, *32*, 429–447.
- (15) Till, M.; Riebniger, D.; Schmitz, H. J.; Schrenk, D. *Chem. Biol. Interact.* **1999**, *117*, 135–150.

Received for review September 28, 2000. Revised manuscript received January 31, 2001. Accepted January 31, 2001.

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